

Imaging and component analysis of pumpkin stem tissue with simultaneous SF-CARS and TPEF microscopy: supplement

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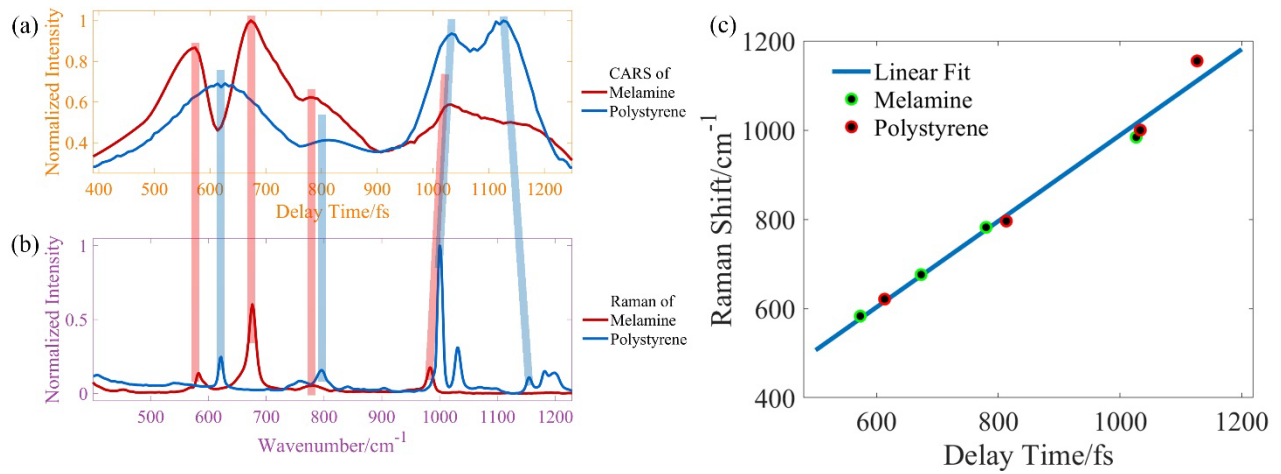
Supplementary Material

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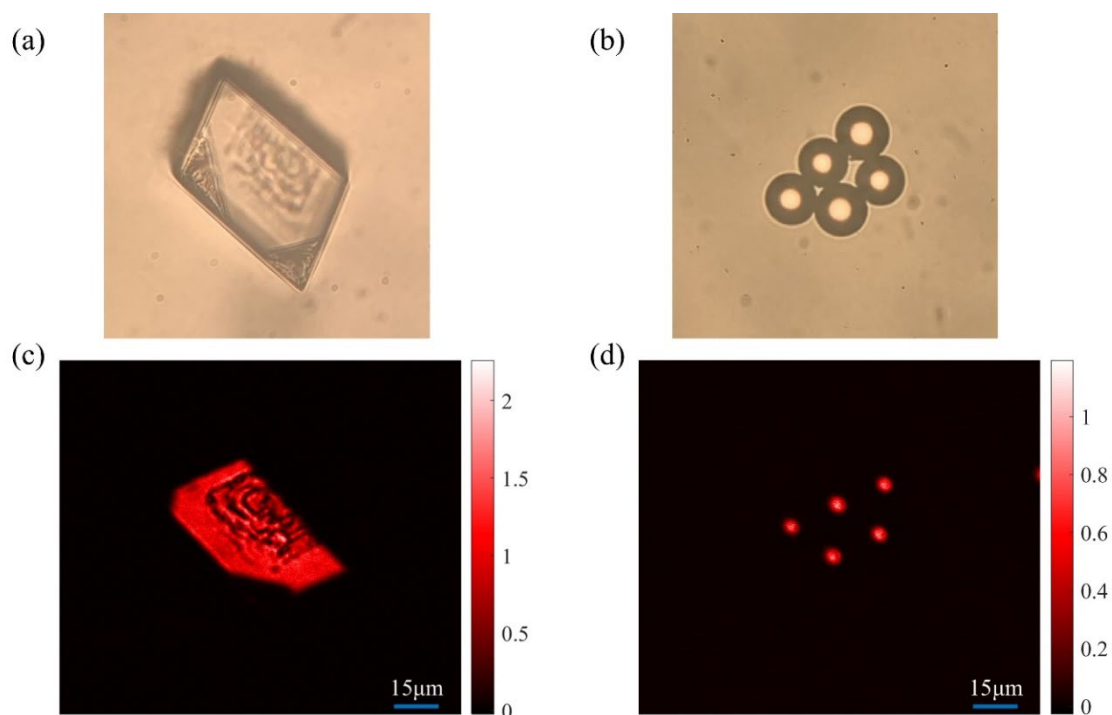
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1 Supplementary Figures



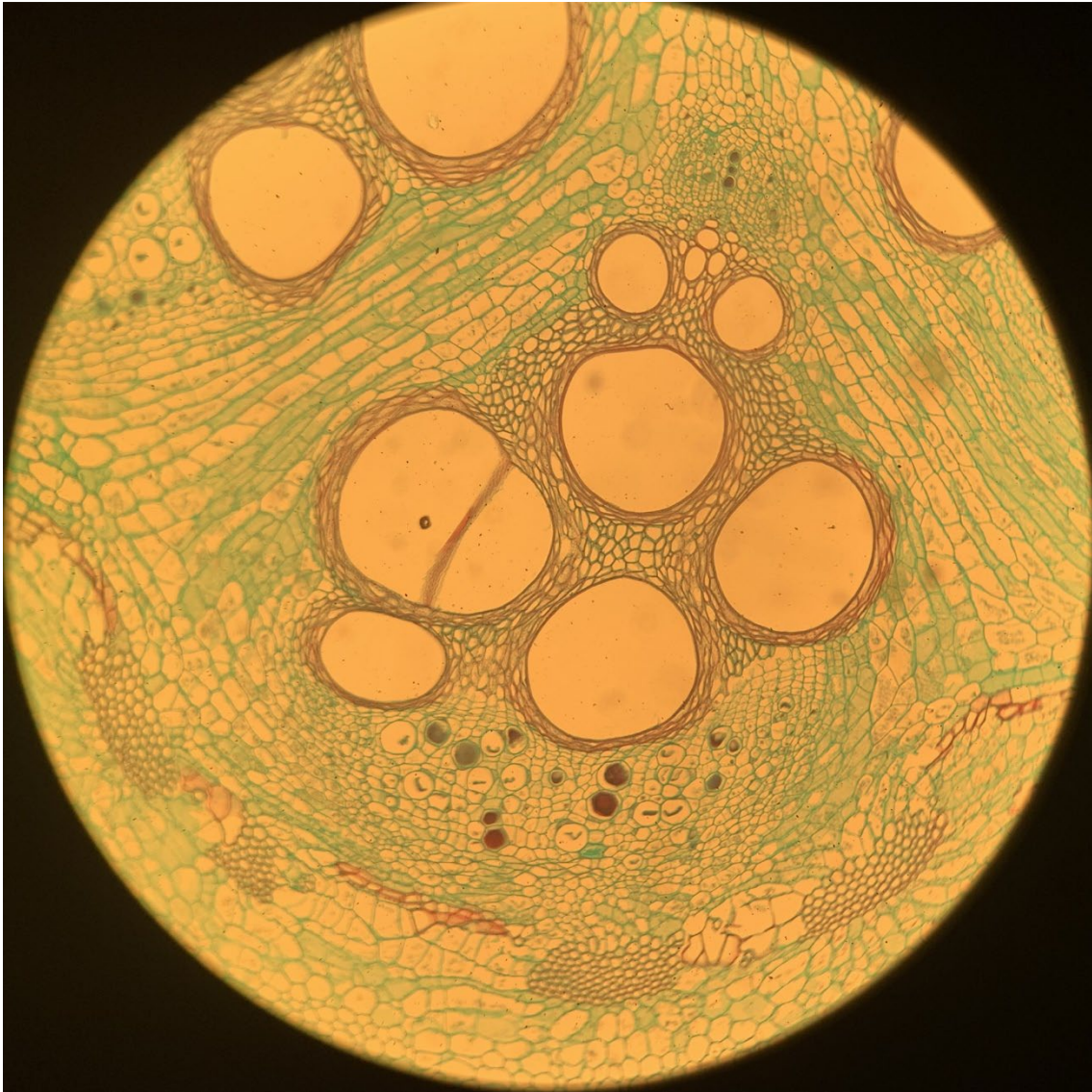
Supplementary Figure 1. The CARS frequency calibration of the SF-CARS microscopy. (a) SF-CARS spectra of pure melamine microcrystal and pure polystyrene beads acquired through tuning the pump-Stokes delay at fixed step of 6.667 fs. (b) The Raman spectra of pure melamine microcrystal and pure polystyrene beads measured with a portable Raman spectrometer (BWS415-785H, B&W Tek). (c) The measured data points from melamine and polystyrene, and the linear fitting model ($R^2=0.9877$).



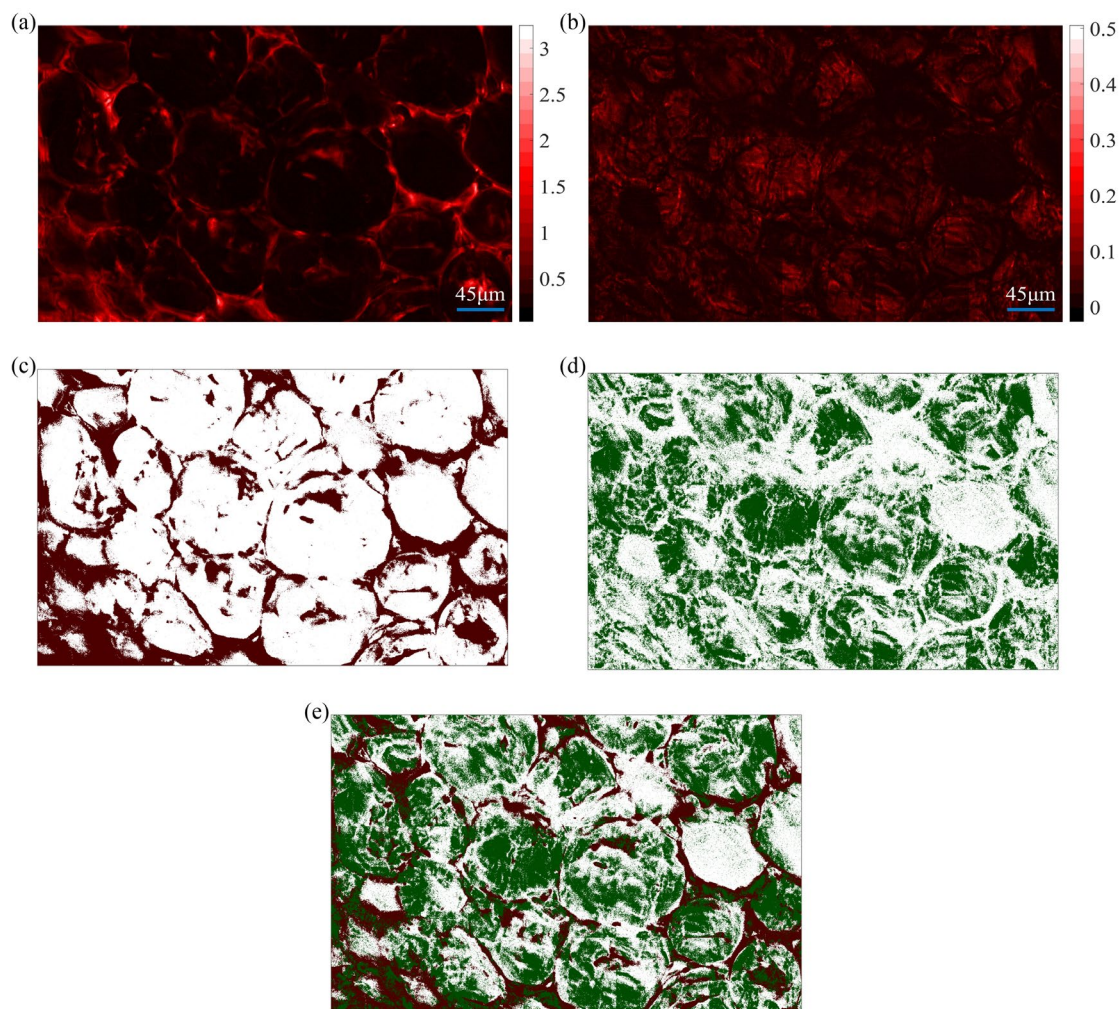
Supplementary Figure 2. The pictures and CARS imaging of pure melamine microcrystal and pure polystyrene beads used in the CARS frequency calibration of the SF-CARS microscopy. (a-b) The pictures of pure melamine microcrystal and pure polystyrene beads acquired with the bright field microscope ($400\times$). (c) The CARS imaging of pure melamine microcrystal at the pump-Stokes delay of 673.33 fs, corresponding to Raman line at 676 cm^{-1} . (d) The CARS imaging of pure polystyrene beads at the pump-Stokes delay of 620 fs, corresponding to Raman line at 622 cm^{-1} .



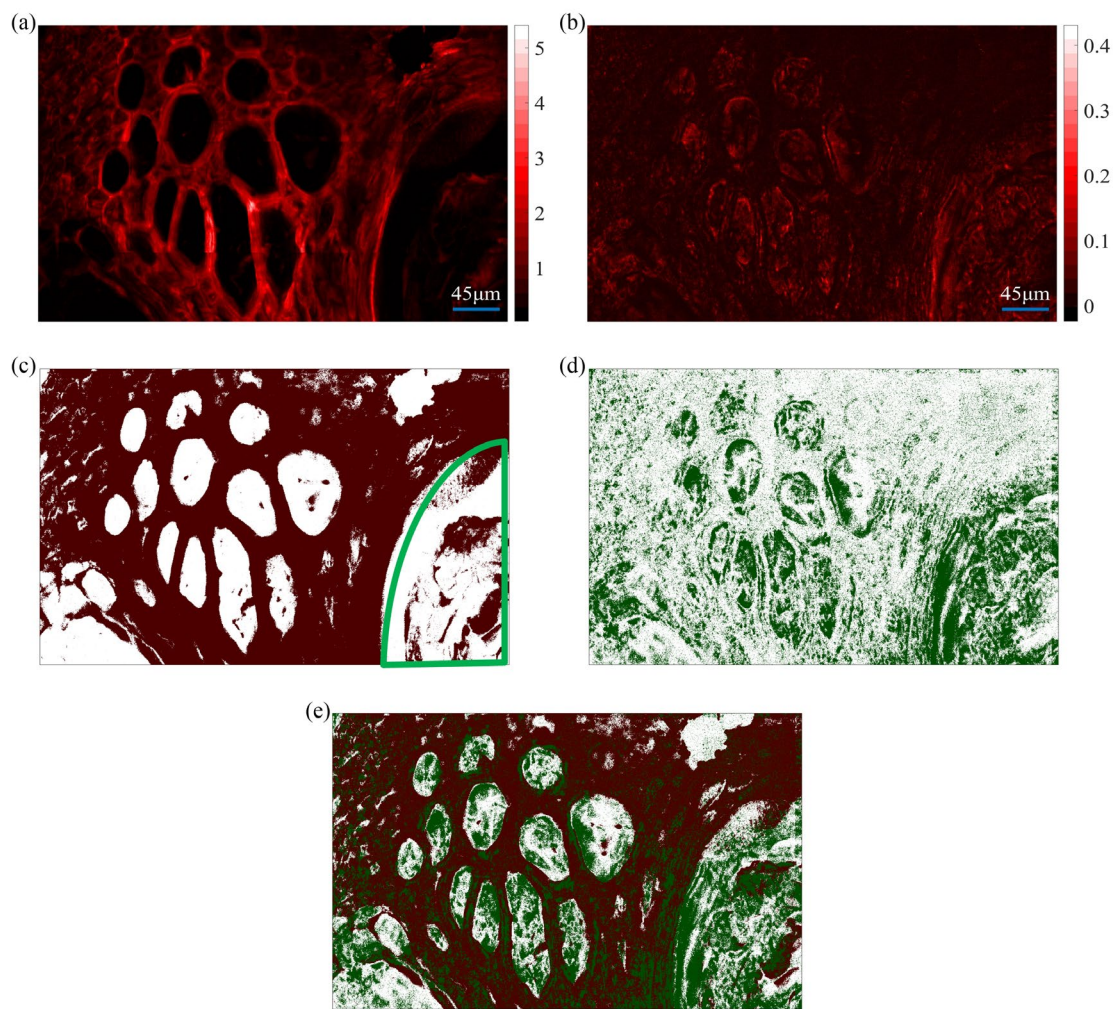
Supplementary Figure 3. The picture of hybrid polystyrene beads acquired with the bright field microscope ($400\times$). Corresponding to Fig. 3 in the manuscript.



- **Supplementary Figure 4.** Picture of the pumpkin stem tissue processed with Safranin-fast green staining and acquired with the bright field microscope. The picture shows clear vessels. Pumpkin stem tissue near vessels is stained to red due to its high lignification degree and pumpkin stem tissue away from vessels is stained to green due to its low lignification degree



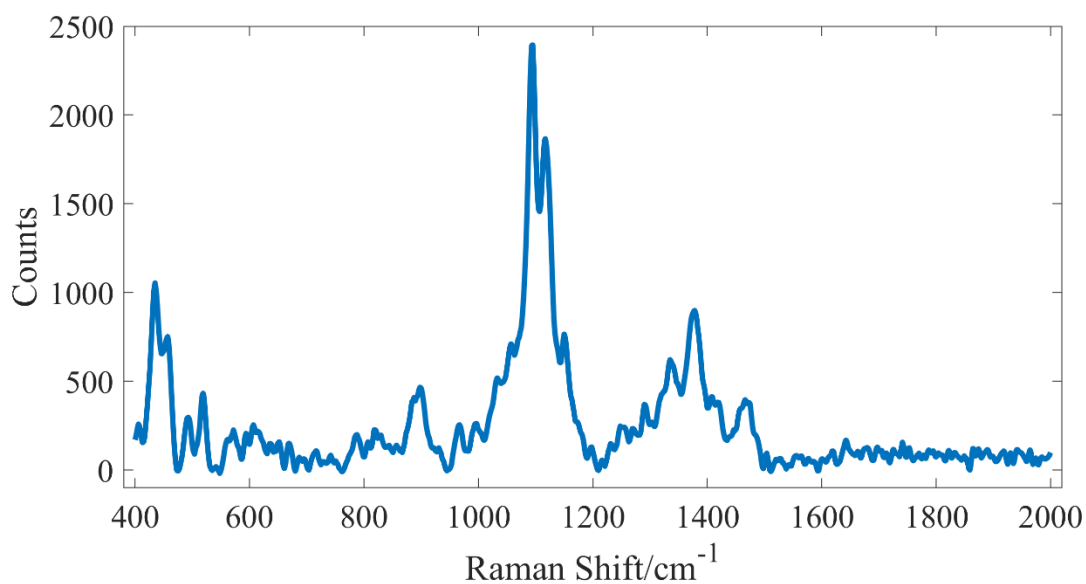
Supplementary Figure 5. Large area ($450\ \mu\text{m} \times 270\ \mu\text{m}$) multimodal nonlinear optical imaging of pumpkin stem tissue away from vessel. (a) CARS imaging. (b) TPEF imaging. (c) Distribution of CARS signal. (d) Distribution of TPEF signal (e) Multimodal nonlinear optical imaging with SF-CARS and TPEF.



Supplementary Figure 6. Large area ($450\ \mu\text{m} \times 270\ \mu\text{m}$) multimodal nonlinear optical imaging of pumpkin stem tissue near vessel. (a) CARS imaging. (b) TPEF imaging. (c) Distribution of CARS signal. (d) Distribution of TPEF signal (e) Multimodal nonlinear optical imaging with SF-CARS and TPEF.



Supplementary Figure 7. (a) The picture of the the pumpkin stem sample prepared for the multimodal nonlinear optical imaging in the manuscript. (b) The outline of the the pumpkin stem sample is indicated with blue lines.



Supplementary Figure 8. The Raman spectrum of pure cellulose microcrystal measured with a portable Raman spectrometer (BWS415-785H, B&W Tek)

2 Supplementary Information

Official website of the supplier of the pumpkin stem tissues: <http://www.dakejx.com/product>.

Website for purchasing the pure PS beads and the PS beads doped with orange fluorescent powder: <https://shop156070021.taobao.com/?spm=2013.1.0.0.246f6d21oLVKMU>.